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Health status and plasma dioxin levels in chloracne cases 20 years after the Seveso, Italy accident

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Summary

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Background The Seveso, Italy accident of 1976 exposed a large population to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or simply dioxin). The accident resulted, mostly among children, in one of the largest ever-reported outbreaks of chloracne, the typical skin disorder due to halogenated-hydrocarbon compounds.
Objectives Approximately 20 years after the accident, we conducted an epidemiological study in Seveso to investigate (a) the health status of chloracne cases; (b) TCDD-chloracne exposure–response relationship; and (c) factors modifying TCDD toxicity.
Methods From 1993 to 1998, we recruited 101 chloracne cases and 211 controls. Trained interviewers administered a structured questionnaire assessing, among other epidemiological variables, information on an extensive list of diseases. During the interview, individual pigmentary characteristics were determined. We measured plasma TCDD levels using high-resolution gas chromatography/mass spectrometry.
Results Plasma TCDD was still elevated (> 10 ppt) in 78 (26·6%) of the 293 subjects with adequate plasma samples, particularly in females, in subjects who had eaten home-grown animals, and in individuals with older age, higher body mass index and residence near the accident site. After 20 years, health conditions of chloracne cases were similar to those of controls from the Seveso area. Elevated plasma TCDD was associated with chloracne [odds ratio (OR) = 3·7, 95% confidence interval (CI) 1·6–8·8, adjusted for age, sex and residence]. Chloracne risk was higher in subjects younger than 8 years at the accident (OR = 7·4, 95% CI 1·8–30·3) and, contrary to previous hypotheses, did not increase at puberty onset or in teenage years. Subjects with elevated TCDD levels and light hair colour had higher relative odds of chloracne (OR = 9·2, 95% CI 2·6–32·5).
Conclusions Dioxin toxicity in chloracne cases was confined to the acute dermatotoxic effects. Chloracne occurrence appeared related to younger age and light hair colour. Age-related dioxin elimination or dilution must be taken into account in interpreting these results.

In July 1976, an industrial accident contaminated a residential area surrounding Seveso, Italy with high levels of the most toxic halogenated hydrocarbon congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, or simply dioxin).[1,2] TCDD, a low-level diffuse contaminant of the environment, is carcinogenic and results in severe neoplastic, developmental, endocrine, immune and reproductive toxicity in experimental animals.[3–5] On the basis of TCDD soil concentration, the

Seveso area was divided into four zones of decreasing levels of contamination: zone A, where the exposure was highest, with 723 inhabitants; zone B with 4281 inhabitants; zone R, with 31 643 inhabitants; and a noncontaminated area surrounding the contaminated zones, which had 181 576 inhabitants.[1] Between September 1976 and February 1978, 193 subjects, 170 of whom (88%) were 14 years of age or younger, were diagnosed with chloracne,[2] the typical skin disorder consequent to intoxication from halogenated hydrocarbons.[6–9]

Chloracne is similar in appearance to acne vulgaris, but it is characterized by pale-yellow keratin cysts and larger and prominent comedones distributed at the malar crescents, postauricular spaces, ears, neck and scrotum.[6,9] Lesions may clear within a few months or persist for over 15 years, usually depending upon severity. Fewer than 4000 cases of chloracne have been reported throughout the world.[10] The Seveso outbreak represents one of the largest documented clusters of chloracne. Less than 0·1% of the subjects in the Seveso cohort, which includes subjects with different degrees of exposure, were diagnosed with chloracne, suggesting that susceptibility or environmental factors may have played a critical role. Although occupational physicians have offered anecdotal reports suggesting that persons of fair complexion are at highest risk for chloracne,[11,12] this hypothesis has never been formally evaluated. Previous studies[12,13] have suggested that chloracne risk may be modified by age, possibly due to interactions between the hormonal changes of puberty and the endocrine-like actions of halogenated hydrocarbons[6,14] or to the occurrence of different patterns of exposure in children.[15]

Approximately 20 years after the accident, we designed a case–control study to collect exposure, epidemiological and clinical data to investigate the association between dioxin and chloracne. We report here data on (a) the health status of chloracne subjects and controls from the same area; (b) possible determinants of dioxin individual levels and susceptibility to dioxin dermatotoxicity; and (c) TCDD-chloracne dose–response relationship and the effect of age on dioxin disposition.

Methods

Between January 1993 and April 1998, we recruited 101 well-documented, previously diagnosed chloracne cases (56 males, 45 females), who had been routinely followed up since the accident, and 211 controls (108 males, 103 females) from the same area. Chloracne diagnoses were made using standard criteria approved by the EEC Commission, based on the type of lesions (comedones, cysts, pustules) and their distribution (face, neck, chest, back, other).[16] Among controls, 101 subjects were matched to chloracne cases by sex, and age and zone of residence at the time of the accident. An additional 110 subjects not affected by chloracne were recruited in a previous study[17,18] and included as controls in the current analyses, to increase the statistical power of the analyses. This second group of controls consisted of a random sample of nonchloracne subjects from the population of the contaminated zones and uncontaminated reference area. Inclusion and exclusion of the second set of control subjects did not substantially modify the findings. We decided to report the results based on the analysis of all subjects ($n = 211$), because the estimates were more precise, with narrower confidence intervals (CIs). A questionnaire including data on demographics, lifestyle, foods consumed at the time of the accident, residential history, and occupations was administered by trained interviewers, who also determined individual pigmentary characteristics. The health status of the study subjects, as well as of their children born after the date of the accident, was ascertained by collecting information on an extensive list of diseases, medication use and reproductive history. We obtained written informed consent from all participants. The local Institutional Review Board reviewed and approved the study. Plasma TCDD was measured at the Centers for Disease Control and Prevention (CDC) using a high-resolution gas chromatographic/mass spectrometric analysis performed on human plasma.[19] Plasma samples from three cases (3·0%) and 16 controls (7·6%) were inadequate for the assay and were excluded from the statistical analyses based on plasma TCDD. Plasma TCDD levels were below detection limit in 163 (55·6%) of the 293 subjects with samples adequate for the dioxin assay. Among them, all but 10 subjects had detection limit below 10 ppt, lipid-adjusted (equivalent to 10 pg g^{-1} fat). We estimated the dioxin levels for these 10 subjects by dividing their detection limit by 2.[20] Subsequently, we divided plasma TCDD in two categories, ≤ 10 ppt or > 10 ppt. The cut-off of 10 ppt is commonly considered to separate background from elevated TCDD plasma levels.[21–24] In univariate analysis, we used Fisher's exact test to assess significance of differences between proportions. We calculated odds ratios (ORs), 95% CIs and tests for trend by means of unconditional logistic regression models, adjusted by age and sex, or by age, sex and zone of residence to take into account the sampling proportions. We performed all analyses using Stata 8.0. All reported P-values are two-sided.

Results

Subjects' characteristics and health status

The chloracne subjects were between 6 months and 46 years of age at the time of the accident (overall median = 8 years; 8 years and 9 years in males and females, respectively) and most of them were prepubescent. Controls were moderately older than cases (age at accident between 3 months and 48 years, median = 8 years, in the first control group; between 4 and 58 years, median = 31 years, in the second control group. Median age was overall 12 years and 17 years in males and females, respectively.) All comparisons between chloracne cases and controls were adjusted for age, in addition to sex and/or zone of residence. Among chloracne cases, 14 women (31·8%) had experienced the menarche and 14 men (25·0%) were 10 years or older at the time of the accident. A

variety of health conditions, diagnosed since the time of the accident until the study period (1993–1998), were reported in the interview-based questionnaire by the study subjects (Table 1). In chloracne cases, the most frequent conditions were allergic (20·8%), gastrointestinal (9·9%) and infectious (8·9%) diseases. Specifically, five chloracne subjects had allergic rhinitis, five had urticaria, six had gastritis, five had herpes infections, and four had hepatitis or jaundice. No cancer diagnosis was reported. Because the controls were older than the chloracne cases, diseases that occur at older age (e.g. osteoarthritis) tended to be more frequent among controls. However, no significant difference was observed between cases and controls in the unadjusted analysis, as well as in the analysis adjusted for age and sex (95% CIs were large, and P-value estimates were not significant). Similarly, no major differences were observed when we compared the frequency of diseases in subjects stratified by exposure to TCDD (data not shown). Seven of the 45 female subjects (15·6%) had irregular menstrual cycles compared with 23 of the 103 female controls (22·3%; P = NS). Twenty-four chloracne subjects had one or more children after the accident, for a total of 20 sons and 11 daughters. The number and sex ratios were not significantly different from those in controls' children (P = NS). None of the children of the chloracne cases had birth defects, cancer, liver disease or diabetes.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin plasma level distribution and determinants of the exposure

Current plasma TCDD ranged from background levels to 475·0 ppt and was higher than 10 ppt in 78 (26·6%) of the 293 subjects with adequate plasma samples. Proximity of primary residence to the site of the accident was a strong determinant of elevated TCDD levels (Table 2). Relative to the individuals in the noncontaminated area, subjects who lived in A zone at the time of the accident were approximately 66 times more likely to have elevated plasma TCDD (OR = 65·9, 95% CI 16·6–262·1 for TCDD > 10 ppt), while those in B and R zones had ORs of 25·8 (95% CI 7·0–95·0) and 3·2 (95% CI 0·5–18·4), respectively (P < 0·001 for trend across zone categories). Plasma TCDD exhibited a nearly linear trend by age. Elevated TCDD levels were approximately six times more

Table 1 Health conditions reported by the chloracne subjects and nonchloracne controls from the Seveso, Italy population[a]

	Chloracne subjects		Control subjects			
			Combined		Exposed (n = 104)[b]	Unexposed (n = 107)[c]
	n (%)	95% CI	n (%)	95% CI	n (%)	n (%)
Allergic diseases	21 (20·8)	13·4–30·0	40 (19·0)	13·9–24·9	20 (19·2)	20 (18·7)
Allergic rhinitis	5 (5·0)	1·6–11·2	17 (8·1)	4·8–12·6	5 (4·8)	12 (11·2)
Asthma	4 (4·0)	1·1–9·8	10 (5·2)	2·3–8·5	5 (4·8)	5 (4·7)
Urticaria	5 (5·0)	1·6–11·2	3 (1·4)	0·3–4·1	2 (1·9)	1 (0·9)
Eczema	4 (4·0)	1·1–9·8	11 (5·2)	2·6–9·1	7 (6·7)	4 (3·7)
Others	8 (7·9)	3·5–15·0	8 (3·8)	1·7–7·3	5 (4·8)	3 (2·8)
Gastointestinal diseases	10 (9·9)	4·9–17·5	20 (9·5)	5·9–14·3	14 (13·5)	6 (5·6)
Gastritis	6 (6·0)	2·2–12·5	5 (2·3)	0·8–5·4	5 (4·8)	0 (0·0)
Peptic ulcer	2 (2·0)	0·2–7·0	1 (0·5)	0·0–2·6	1 (1·0)	0 (0·0)
Others	2 (2·0)	0·2–7·0	14 (6·6)	3·8–10·9	8 (7·7)	6 (5·6)
Infectious diseases	9 (8·9)	4·1–16·2	16 (7·6)	4·4–12·0	7 (6·7)	9 (8·4)
Herpes labialis/zoster	5 (5·0)	1·6–11·2	12 (5·7)	3·0–9·7	7 (6·7)	5 (4·7)
Hepatitis/jaundice	4 (4·0)	1·1–9·8	2 (1·0)	0·1–3·4	1 (1·0)	1 (0·9)
Others	0 (0·0)	–	3 (1·4)	0·3–4·1	0 (0·0)	3 (1·4)
Endocrine diseases	5 (5·0)	1·6–11·2	6 (2·8)	1·1–6·1	4 (3·9)	2 (1·9)
Thyroid diseases	2 (2·0)	0·2–7·0	2 (1·0)	0·1–3·4	1 (1·0)	1 (0·9)
Diabetes	1 (1·0)	0·0–5·4	2 (1·0)	0·1–3·4	1 (1·0)	1 (0·9)
Others	2 (2·0)	0·2–7·0	2 (1·0)	0·1–3·4	2 (1·9)	0 (0·0)
Respiratory diseases	2 (2·0)	0·2–7·0	14 (6·6)	3·7–10·9	11 (10·6)	3 (2·8)
Bronchitis	0 (0·0)	–	6 (2·8)	1·1–6·1	6 (5·8)	0 (0·0)
Pneumonia/pleurisy	0 (0·0)	–	4 (1·9)	0·5–4·8	4 (3·9)	0 (0·0)
Others	2 (2·0)	0·2–7·0	7 (3·3)	1·3–6·7	4 (3·9)	3 (2·8)
Miscellaneous	11 (10·9)	5·6–18·7	39 (18·5)	13·5–24·4	22 (21·2)	17 (15·9)
Anaemia	3 (3·0)	0·6–8·4	9 (4·3)	2·0–7·9	4 (3·9)	5 (4·7)
Osteoarthritis	2 (2·0)	0·2–7·0	17 (8·1)	4·8–12·6	11 (10·6)	6 (5·6)
Psoriasis	2 (2·0)	0·2–7·0	3 (1·4)	0·3–4·1	2 (1·9)	1 (0·9)
Others	5 (5·0)	1·6–11·2	12 (5·6)	3·0–9·7	6 (5·8)	6 (5·6)

[a]Diseases diagnosed from the date of the accident (10 July 1976) to the study period (1993–1998). Subjects may have had more than one illness after the accident and may appear in one or more disease category. [b]Exposed zones (A + B + R). [c]Unexposed zones (non-A + B + R).

Table 2 Determinants of elevated (> 10 ppt) plasma levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) measured in subjects from the Seveso population from 1993 to 1998[a]

	Plasma TCDD				
	≤ 10 ppt (N = 215) n (%)	> 10 ppt (N = 78) n (%)	OR[b]	95% Ci[b]	P-value[b]
Residence at the date of the accident					
Noncontaminated	73 (34·0)	4 (5·1)	1·0	–	–
R zone	73 (34·0)	3 (3·9)	3·2	0·5–18·4	0·20
B zone	36 (16·7)	27 (34·6)	25·8	7·0–95·0	< 0·001
A zone	33 (15·3)	44 (56·4)	65·9	16·6–262·1	< 0·001
Age at the date of the accident					
6 months to 8 years	89 (41·4)	16 (20·5)	1·0	–	
8–13 years	60 (27·9)	10 (12·8)	1·8	0·6–5·1	0·27
13–18 years	13 (6·1)	15 (19·2)	3·6	1·1–11·0	0·03
> 18 years	53 (24·7)	37 (47·4)	5·9	2·3–15·1	< 0·001
Sex					
Male	132 (61·4)	21 (26·9)	1·0	–	
Female	83 (38·6)	57 (73·1)	5·3	2·5–11·2	< 0·001
Body mass index[c]					
< 21·7 kg m^{-2}	75 (35·1)	22 (28·6)	1·0	–	
21·7–25 kg m^{-2}	69 (32·2)	26 (33·8)	1·6	0·6–4·0	0·29
> 25 kg m^{-2}	70 (32·7)	29 (37·7)	2·8	1·0–4·0	0·04

[a]Lipid-adjusted plasma TCDD levels measured approximately 20 years after the Seveso accident. [b]Odds ratios (ORs), 95% confidence intervals (CIs) and P-values adjusted in multiple regression analysis for sex, age and zone of residence at the date of the accident. [c]Body mass index measured at the interview. Total number of subjects varies because of missing values.

frequent (OR = 5·9; 95% CI 2·3–15·1) in subjects older than 18 years at the time of the accident relative to individuals younger than 8 years (P < 0·001 for trend across age categories). As expected,[17] women had higher plasma TCDD levels than men (OR = 5·3, 95% CI 2·5–11·2). Occurrence of elevated TCDD levels increased with increasing body mass index (BMI) (OR = 1·6, 95% CI 0·6–4·0 for BMI 21·7–25 kg m^{-2} and OR = 2·8, 95% CI 1·0–4·0 for BMI > 25 kg m^{-2}, relative to BMI < 21·7 kg m^{-2}; P = 0·04 for trend across BMI categories). All statistical models were adjusted for age, sex, and zone of residence.

We evaluated whether personal behaviour and events at the time of the accident were associated with elevated dioxin levels. Lower plasma TCDD levels were found among residents of the study area who were reported to have been on vacation at the date of the accident (OR = 0·1, 95% CI 0·03–0·7 for plasma TCDD > 10 ppt). Subjects who had eaten home-grown poultry or livestock had higher odds of elevated plasma TCDD (OR = 4·9; 95% CI 2·0–11·7). Similarly, subjects who had direct experience of the accident (i.e. saw or smelt the toxic cloud, heard the explosion, experienced eye/throat irritation or itching of the skin), and those who recalled the details of the accident (e.g. they could correctly remember the date and/or the time of the accident) had higher TCDD plasma levels in the analyses adjusted by age and sex. However, this association was not statistically significant when we adjusted the models also for zone of residence.

Chloracne and plasma 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels

Chloracne was nearly four times more frequent in subjects with current plasma TCDD > 10 ppt (OR = 3·7, 95% CI 1·6–8·8, adjusted for zone of residence and age at the accident and sex) (Table 3). Among them, 21 chloracne cases and 12 controls had TCDD plasma levels higher than 50 ppt and an OR for chloracne of 20·4 (95% CI 5·1–81·0), relative to subjects with TCDD ≤ 10 ppt.

Subjects with TCDD levels > 10 ppt who were 8 years of age or younger at the time of the accident had OR = 7·4 (95% CI 1·8–30·3) for chloracne (Table 3). Among subjects older than 8 years, the OR was 1·3 (95% CI 0·6–3·1) (P = 0·02 for the interaction between age and plasma TCDD).

We evaluated whether subjects' pigmentary characteristics were related to chloracne. Chloracne status exhibited borderline association with eye colour (OR = 1·6, 95% CI 0·9–2·8 for light brown, green, grey or blue vs. dark brown; adjusted for age, sex and zone of residence), while skin colour (OR = 0·7, 95% CI 0·4–1·2 for light vs. medium or dark), hair colour (OR = 0·9, 95% CI 0·5–1·7 for light brown, reddish brown, blonde or red vs. dark brown or black) or skin reaction after 1 h in the sun (OR = 1·5, 95% CI 0·7–2·9 for subjects who tended to burn vs. never burn) were not associated with the disease. Interestingly, subjects with TCDD levels > 10 ppt had higher relative odds of chloracne if they had light

Table 3 Relative odds of chloracne for subjects with elevated (> 10 ppt) current plasma levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), measured in subjects from the Seveso population from 1993 to 1998[a]

	Chloracne subjects (n = 98)	Control subjects (n = 195)	OR[b]	95% CI[b]
All subjects				
Plasma TCDD ≤ 10 ppt	70	145	1·0	–
Plasma TCDD > 10 ppt	28	50	3·7	1·6–8·8
Age at the accident ≤ 8 years				
Plasma TCDD ≤ 10 ppt	38	51	1·0	–
Plasma TCDD > 10 ppt	12	4	7·4[c]	1·8–30·3
Age at the accident > 8 years				
Plasma TCDD ≤ 10 ppt	32	94	1·0	–
Plasma TCDD > 10 ppt	16	46	1·3[c]	0·6–3·1
Dark hair colour				
Plasma TCDD ≤ 10 ppt	43	65	1·0	–
Plasma TCDD > 10 ppt	14	23	2·1[d]	0·7–6·1
Light hair colour				
Plasma TCDD ≤ 10 ppt	25	67	1·0	–
Plasma TCDD > 10 ppt	13	17	9·2[d]	2·6–32·5

[a]Lipid-adjusted plasma TCDD levels measured approximately 20 years after the accident. [b]Odds ratios (ORs) and 95% confidence intervals (CIs) computed using logistic regression models with plasma TCDD, sex, age and zone of residence at the date of the accident as independent variables. [c]P = 0·02 for the difference between age-specific ORs (likelihood ratio test for the interaction between age and plasma TCDD levels). Eight years was the median age of the chloracne cases at the date of the accident. [d]P = 0·04 for the difference between hair-colour-specific ORs (likelihood ratio test for the interaction between hair colour and plasma TCDD levels). Current dark hair colour: black or dark brown; light hair colour: light brown, reddish brown, blonde or red.

(OR = 9·2, 95% CI 2·6–32·5) rather than darker hair colour (OR = 2·1, 95% CI 0·7–6·1, P = 0·04 for the interaction between plasma TCDD and hair colour), when compared with those with lower TCDD levels (Table 3).

Discussion

In the present study, we found a strong association between TCDD levels and chloracne occurrence, which persisted after 20 years from the exposure. This was possible because of the long half-life of dioxins in humans.[25–27] Some investigators have suggested that chloracne is a 'sentinel health effect' and no effects relevant to the health of the exposed subjects may occur in its absence.[28–30] Others have proposed that chloracne is associated with very intense exposures, well above those necessary to elicit other systemic effects.[6,9,31,32] We assessed whether occurrence of diseases in chloracne subjects, who experienced intense dioxin exposure, had been higher than in controls after the accident. Even though several medical conditions were reported by the chloracne cases, frequency and type of disorders did not significantly differ between cases and controls during the 20-year life span we documented. Thus, dioxin toxicity in these subjects appears to be confined to the acute dermatotoxic effects. Our results were based on a relatively limited statistical power to detect the association with specific diseases, as well as to test for the interaction of plasma TCDD with possible modifiers of TCDD toxicity. However, this study is unique with regard to age and sex distribution of the study subjects, relatively pure exposure to TCDD, availability of epidemiological and clinical data, as well as individual assessment of TCDD plasma levels.

In our study, dioxin levels measured 20 years after the exposure appeared to be positively associated with age at the time of the accident. This is in contrast with what was found in samples collected in Seveso at the time of the accident.[15] In fact, in those samples TCDD levels were highest among the youngest subjects and decreased until approximately 13 years of age, after which there was no change.[15] We found a nearly linear increase of dioxin levels by age, within the age range of our study subjects (Table 2). Differential elimination by age or body dilution of dioxin consequent to body growth in young children[33] may explain this discrepancy. This finding is important for interpreting the association of dioxin with chloracne. In our study, chloracne occurrence was more strongly associated with plasma TCDD levels among individuals who were younger than 8 years at the time of the accident (OR = 7·4, Table 3). This may reflect a higher elimination/dilution of TCDD, as well as an increased sensitivity to chloracnegenic factors in younger subjects. The hypothesis that the endogenous hormonal changes of puberty may favour chloracne development[6] was not supported by our results. In fact, the majority of the chloracne cases were prepubescent at the time of the accident, and the highest risk was associated with age younger than 8 years.

We found a strong association between plasma TCDD levels and chloracne among subjects with light hair colour. In addition, subjects with light eye colour exhibited a suggestive, although nonsignificant, increased risk of chloracne. Genetic

or environmental factors related to pigmentation may modify TCDD toxicity. The Seveso accident occurred in mid summer, and fair pigmentation results in low protection against UV radiation. One might speculate, for example, that UV exposure modifies susceptibility to chloracne in fair-skinned subjects. However, individuals with light skin colour, as well as those who tended to burn after short sun exposure, were not at increased risk of chloracne in comparison with subjects with darker pigmentation. This discrepancy may be due to misclassification of skin pigmentation. In fact, skin colour assessment is highly subjective[34] and skin reaction to sun was self-reported. Thus, our finding requires confirmation in larger studies, possibly with standardized measurements of pigmentation characteristics.

In conclusion, 20 years after the Seveso accident, dioxin levels were still elevated in exposed individuals, particularly in females, in subjects who had eaten home-grown animals, and in individuals with older age, higher BMI and residence near the accident site. Plasma dioxin was strongly associated with chloracne. This association was modified by age and pigmentation characteristics, possibly reflecting an increased sensitivity to chloracnegenic factors or differential dioxin clearance. TCDD toxicity in chloracne subjects appeared to be confined to the acute dermatotoxic effects.

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